

# Conditions necessary for photosynthesis

This practical can be used to easily assess Scientific enquiry standards 1, 3 and 4.

Relevant biology standards: 11A 7.1 & 8.4 and 12F 7.1 & 8.4

## **Materials:**

### For setting up:

Potted plant (e.g. hibiscus, geranium/pelargonium)

Sodium hydroxide pellets

Sellotape

Polythene bag

Black paper or aluminium foil

Plant with variegated leaves

### For testing leaves for starch:

Boiling water bath

Meths or ethanol

Boiling tube

Test tube holder

Test tube rack

Petri dish or glass/ceramic tile

Iodine solution

## **Procedure:**

**Destarch** the plant by placing it in the dark for 72 hours (over a weekend is good). Small plants may be placed in a cupboard, large plants can be covered by a polythene bag. The entire plant needs to be covered, otherwise the products of photosynthesis may be **translocated** from photosynthesizing parts of the plant to the covered parts of the plant, and the destarching will be ineffective.

Three experiments are usually conducted:

**1. Is chlorophyll necessary for photosynthesis?**

Select a **variegated** plant for this. Variegated leaves do not need to be destarched, since no starch is formed in the absence of chlorophyll.

Hypothesis: Photosynthesis cannot occur where there are no chloroplasts.

**2. Is carbon dioxide necessary for photosynthesis?**

Encase some leaves in a transparent plastic bag containing pellets of sodium hydroxide. Seal the bag around the petiole or stem.

Hypothesis: NaOH absorbs atmospheric  $CO_2$ . Photosynthesis cannot occur in the absence of the raw material  $CO_2$ .

**3. Is sunlight necessary for photosynthesis?**

Cover part of a leaf with black paper or aluminium foil.

Hypothesis: Photosynthesis cannot occur in the absence of sunlight, i.e. only those parts of the leaf exposed to sunlight will show the presence of starch.

## Testing the leaves for starch:

- Bring some water to boiling either in a beaker of water heated by a Bunsen burner or in an electric **water bath**.

- Briefly immerse the leaf in the boiling water to kill it.

Student question: Bubbles of gas form on the undersurface of the leaf when we put it in boiling water - why?

Answer: The expanding air inside the leaf comes out through the stomata which are only located on the lower epidermis of a dicot leaf.

- Place this leaf in a **boiling tube with** enough **alcohol** to cover the leaf. Place the boiling tube in the boiling **water bath**.

Safety tip: When the alcohol begins to boil, turn off the Bunsen. This will prevent the alcohol from boiling too vigorously and shooting out of the mouth of the boiling tube.

- The purpose of the alcohol is to dissolve the waxy layer covering the leaf and dissolve the chlorophyll in the leaf. If the alcohol stops boiling and the leaf is still green, carefully bring the alcohol to the boil again. **Continue boiling the leaf until the green colour is removed from the leaf and the leaf is pale yellow.**
- Remove the leaf from the alcohol and **dip it in the hot water bath**. The leaf becomes brittle in alcohol, so **this softens it** again.
- Spread the leaf on a tile or Petri dish and **irrigate with iodine** solution. The black colour develops slowly, but is expected in areas which were able to photosynthesize.

## Additional notes:

Avoid using very rigid or waxy leaves. These seldom decolourise properly, and iodine solution will not penetrate the leaf properly.

Run the experiment for several hours in bright sunlight. This should be sufficient time for starch to be deposited in the leaves. If the experiment is continued for too long, translocation of sugars may occur to those parts of the plant which aren't supposed to be able to manufacture starch, and a false positive result may be indicated.

### Steps in the experiment:

In this example, potted basil was used.

The plant was destarched by leaving it in a cupboard under the sink from Thursday afternoon to Sunday morning.

#### **a) To test whether carbon dioxide is necessary for photosynthesis:**



Twig encased in a polythene bag.  
Sunlight can reach the leaves.

NaOH absorbs CO<sub>2</sub>

The plant was placed outside for 3 hours.



The entire twig was harvested after 3 hours and the leaves were killed by dipping in boiling water.

The leaves were placed in ethanol, which was put in the water bath and brought to boiling.

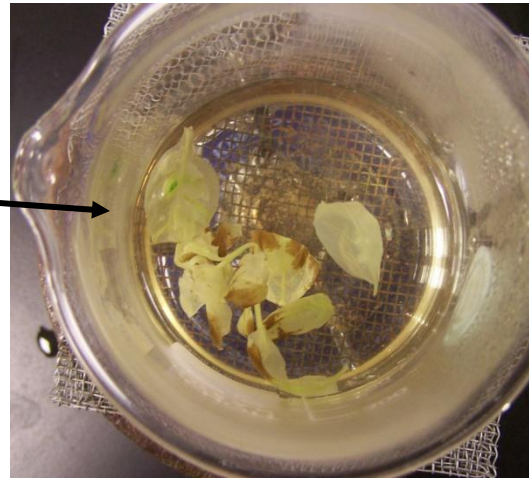




The chlorophyll is dissolved by the alcohol and the leaves eventually lost all green colour.

The alcohol solution was poured off and the leaves were tipped into the water bath to wash and soften them.

(Brown patches indicate scorching from leaving the plant outside on an excessively hot day)



After flooding with iodine solution, black colour developed where starch had been deposited. Although some leaves showed the presence of starch, the leaves at the most distal part of the shoot contained little or no starch. This would seem to confirm the hypothesis that  $\text{CO}_2$  is necessary for photosynthesis.



**b) To test if sunlight is necessary for photosynthesis:**



The leaves were partly covered with black paper to block sunlight, then exposed for 3 hours.

It was expected that only the exposed parts of the leaf would show the presence of starch.

The exposed parts of the leaf form the control for this experiment.

Aluminium foil is a good alternative to black paper as it reflects radiation and is completely opaque.

**c) To test if chlorophyll is necessary for photosynthesis:**



Variegated leaves clearly show that the distribution of starch within the leaf corresponds with the distribution of chlorophyll.

original leaf



after boiling  
in alcohol



after flooding with  
iodine

